Determination of Bedaquiline in Human Serum Using Liquid Chromatography-Tandem Mass Spectrometry

Alffenaar, Jan-Willem C.; Bolhuis, Mathieu; van Hateren, Kai; Sturkenboom, Marieke; Akkerman, Onno; de Lange, Wiel; Greijdanus, Ben; van der Werf, Tjip; Touw, Daan

Published in:
Antimicrobial Agents and Chemotherapy

DOI:
10.1128/AAC.00276-15

AAC Journals.ASM.org

Determination of Bedaquiline in Human Serum Using Liquid Chromatography-Tandem Mass Spectrometry

Jan-Willem C. Alffenaar,[a] Mathieu Bolhuis,[a] Kai van Hateren,[a] Marieke Sturkenboom,[a] Onno Akkerman,[b] Wiel de Lange,[b] Ben Greijdanus,[a] Tjip van der Werf,[b,c] Daan Touw[a,d]

University of Groningen, University Medical Center Groningen, Department of Clinical Pharmacy and Pharmacology, Groningen, The Netherlands[a]; University of Groningen, University Medical Center Groningen, Department of Pulmonary Diseases and Tuberculosis, Groningen, The Netherlands[b]; University of Groningen, University Medical Center Groningen, Department of Internal Medicine, Groningen, The Netherlands[c]; University of Groningen, University Center of Pharmacy, Section of Pharmacokinetics, Toxicology and Targeting, Groningen, The Netherlands[d]

Bedaquiline, a diarylquinoline for the treatment of multidrug-resistant tuberculosis (TB), relies on exposure-dependent killing. As data on drug exposure in specific populations are scarce, pharmacokinetic studies may be of interest. No simple and robust validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method has been reported to date. Therefore, a new method using a quadrupole mass spectrometer was developed for analysis of bedaquiline and *N*-monodesmethyl bedaquiline (M2) in human serum, using deuterated bedaquiline as the internal standard. The calibration curve was linear over a range of 0.05 (lower limit of quantification [LLOQ]) to 6.00 mg/liter for both bedaquiline and M2, with correlation coefficient values of 0.997 and 0.999, respectively. The calculated accuracy ranged from 1.9% to 13.6% for bedaquiline and 2.9% to 8.5% for M2. Within-run precision ranged from 3.0% to 7.2% for bedaquiline and 3.1% to 5.2% for M2, and between-run precision ranged from 0.0% to 4.3% for bedaquiline and 0.0% to 4.6% for M2. Evaluation of serum concentrations in a patient receiving bedaquiline showed high levels at the end of treatment, reflecting accumulation of the drug. More observational pharmacokinetic data are needed to relate altered drug concentrations to clinical outcome or adverse drug effects. A simple LC-MS/MS method to quantify bedaquiline and M2 levels in human serum using a deuterated internal standard has been validated. This method can be used in clinical studies and daily practice.

Bedaquiline is a diarylquinoline and was approved by the United States Food and Drug Administration (FDA) in 2012 under the accelerated-approval program. This drug is indicated for treatment of multidrug-resistant pulmonary tuberculosis (MDR-TB) in combination with other active anti-TB drugs in adults without other treatment options (1). This new anti-TB drug, formally known as TMC207, showed exposure-dependent killing of *Mycobacterium tuberculosis* (2, 3). The drug is metabolized by cytochrome P 450 enzyme 3A4 (CYP3A4) to its 5-fold-less-active metabolite *N*-monodesmethyl bedaquiline (M2) (4).

Bedaquiline therapy is started in a loading dose of 400 mg once daily for 2 weeks followed by 200 mg 3 times per week for 22 weeks. This dosing regimen was chosen because pharmacokinetics (PK) modeling predicted gradual accumulation in plasma and tissues (4).

At this time, little data on PK in patients with advanced MDR-TB or suffering from comorbidities are available apart from the data that were collected in the clinical studies (4). However, variability in the PK of antituberculosis drugs is considerable and altered drug exposure may translate to variations in clinical outcomes (5, 6). Clearly, there is a need for more-descriptive (population) PK studies on bedaquiline to determine predictors of exposure, drug-drug interaction (7), and PK-pharmacodynamics (PD) relationships. The World Health Organization has released a guide for the use of bedaquiline and encourages additional evaluation of this new drug (8).

As bedaquiline serum concentrations may be altered in MDR-TB patients with comorbidities, a suitable method of analysis is needed. Only advanced analytical methods using high-performance liquid chromatography (HPLC) inductively coupled plasma (ICP) mass spectrometry (MS) have been presented previously (9, 10). These methods were used for metabolite profiling of bedaquiline in feces. Due to their advanced setup requirements, these analytical procedures are generally not available in clinical laboratories that perform routine monitoring of serum concentrations of anti-TB drugs. In addition, despite their advanced setup, the results determined with the combination of HPLC and ICP are not as specific as those determined with tandem mass spectrometry (MS/MS), as it measures only the bromine. Simpler analytical methods using liquid chromatography coupled to tandem mass spectrometry are described in reports of the clinical studies, but the data lack the detail necessary to be useful for clinical laboratories as guidance to develop their own analytical methods (11). Therefore, the purpose of this study was to develop a simple and robust LC-MS/MS method without extensive sample processing, using deuterated bedaquiline as the internal standard, to quantify bedaquiline and M2 serum concentrations in MDR-TB patients.

Received 3 February 2015 **Returned for modification** 14 March 2015
Accepted 28 June 2015

Accepted manuscript posted online 6 July 2015

Citation Alffenaar J-WC, Bolhuis M, van Hateren K, Sturkenboom M, Akkerman O, de Lange W, Greijdanus B, van der Werf T, Touw D. 2015. Determination of bedaquiline in human serum using liquid chromatography-tandem mass spectrometry. Antimicrob Agents Chemother 59:5675–5680. doi:10.1128/AAC.00276-15.

Address correspondence to Jan-Willem C. Alffenaar, j.w.c.alffenaar@umcg.nl.

doi:10.1128/AAC.00276-15

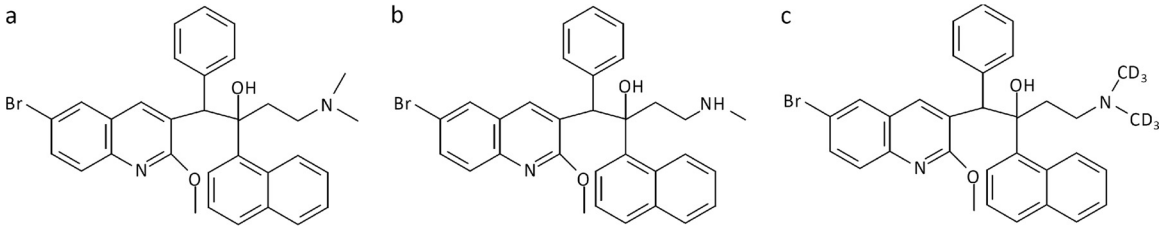

FIG 1 Chemical structures. Structure a, bedaquiline; structure b, M2; structure c, D6-bedaquiline.

MATERIALS AND METHODS

Analysis. Bedaquiline, M2, and D6-bedaquiline (internal standard) (Fig. 1) were obtained from Janssen Pharmaceuticals (Beerse, Belgium). Water, acetonitrile, ammonium acetate, and acetic acid were all of suitable analytical grade. Pooled human serum samples were made available according to the guidelines of the University Medical Center Groningen.

Bedaquiline stock solutions of 500 mg/liter of dimethyl sulfoxide (DMSO)–methanol (50:50) were prepared separately and used for the preparation of calibration standards and quality control (QC) samples using blank human serum. The amounts of stock solution added to the serum did not exceed 5% of the total volume. Calibration standards and QC samples were freshly prepared on the first validation day and stored at −20°C for use on the other validation days. Concentrations of the standards and QC samples are listed in Table 1. The internal standard solution was prepared by diluting a 500 mg/liter bedaquiline-D6 stock solution to 0.1 mg/liter with methanol-acetonitrile (precipitation reagent) (160:840).

Samples were prepared by adding 750 μl precipitation reagent to 10 μl serum and subsequent vortex mixing for 1 min. The vials were centrifuged for 5 min at 11,000 rpm, and 5 μl of the upper layer was injected into the LC-MS/MS system.

The analysis was performed on a triple-quadrupole LC-MS/MS system (Thermo Scientific, San Jose, CA, USA) with a MS Pump Plus and Surveyor autosampler (Finnigan). The mass spectrometer was a TSQ Quantum Access Max mass spectrometer. The temperature of the autosampler was set at 10°C. A HyPURITY C_{18} analytical column (Thermo Scientific, Interscience, Breda, The Netherlands) (50 by 2.1 mm; pore size, 3 μm) was used for chromatographic separation, and the temperature was set at 20°C. The mobile phase had a flow rate of 500 μl/min and consisted of purified water, acetonitrile, and an aqueous buffer (containing ammonium acetate [10 g/liter], acetic acid [35 mg/liter], and trifluoroacetic anhydride [2 ml/liter] in water). The elution gradient is shown in Table 2. The method had a run time of 2.6 min.

The MS system was configured using the positive electrospray ionization mode and selected reaction monitoring (SRM) with a spray voltage of 3,500 V, a capillary temperature of 350°C, and sheath gas and auxiliary pressures of 35 and 10 arbitrary units, respectively. Mass transitions for bedaquiline were 555.1 *m/z* to 58.4 *m/z*, for D6-bedaquiline were 561.2 *m/z* to 64.4 *m/z*, and for M2 were 541.1 *m/z* to 480.1, using a scan width of 0.5 *m/z*. Collision energy was determined at 34 electron volts (eV) for both transitions. Xcalibur software version 2.0.7 (Thermo Fisher, San Jose, CA, USA) was applied for peak height and peak area integration for all components.

Analytical method validation. The criteria for validation of the method included selectivity and sensitivity (i.e., determination of the lower limit of quantitation [LLOQ]), linearity, accuracy and precision, recovery, matrix effect, process efficiency, and dilution integrity. The calibration curve for bedaquiline consisted of 8 samples with concentrations of 0.05, 0.25, 0.50, 1.00, 2.00, 4.00, 5.00, and 6.00 mg/liter. Quality control (QC) samples with 4 different concentrations of bedaquiline were used, where the LLOQ was 0.05 mg/liter, the low concentration was 0.25 mg/liter, the medium concentration was 2.50 mg/liter, and the high concentration was 5.00 mg/liter. The effect of endogenous substances was examined by an infusion ion suppression test using 6 lots of different blank serums, and the results were compared to the response seen at the LLOQ. During 3 days, a single calibration curve was analyzed each day and accuracy was measured by evaluation of five determinations per QC sample on three consecutive days. Precision was divided into within-run and between-run data using the same method as described above for the accu-

TABLE 1 Validation results

Criterion	Value at indicated QC concn level			
	LLOQ	Low	Medium	High
Nominal concn (mg/liter)				
Bedaquiline	0.05	0.25	2.5	5
M2	0.05	0.25	2.5	5
Accuracy (bias [%])				
Bedaquiline	13.6	2.9	1.9	4.2
M2	8.5	2.9	5.4	5.9
Within-day precision (CV [%])				
Bedaquiline	7.2	5.1	3.0	3.0
M2	4.4	3.2	3.6	3.1
Between-day precision (CV [%])				
Bedaquiline	4.3	0.0[a]	2.5	0.0[a]
M2	4.6	0.0[a]	1.4	2.6
Matrix effect (bias [%])				
Bedaquiline		103.0	103.5	98.5
M2		100.8	96.6	95.0
Recovery (bias [%])				
Bedaquiline		103.5	98.4	106.8
M2		99.7	101.7	103.9
Total process efficiency (bias [%])				
Bedaquiline		106.6	101.8	105.2
M2		100.5	98.3	98.7

[a] No additional variation was added to the intraday imprecision value as a result of measurements on separate days.

TABLE 2 Gradient elution

Time (min)	% elution[a]		
	A	B	C
0.00	5	95	0
1.50	5	0	95
2.20	5	0	95
2.21	5	95	0
2.60	5	95	0

[a] A, ammonium acetate 5.0 g/liter, acetic acid 100% 25 ml/liter, trifluoroacetic acid (99.95%) 2 ml/liter; B, ultrapure water; C, acetonitrile LC-MS (Biosolve).

racy determinations. All three calibration curves were assessed with the use of Analyze-it to assess the linear regression of the 8-point calibration curve. For each accuracy and precision determination, concentration bias and coefficient of variation (CV) were calculated per run and each value was not allowed to exceed 20% for LLOQ and 15% for the other QC levels. Within-run, between-run, and overall CV values were calculated with the use of one-way analysis of variance (ANOVA).

Procedures to evaluate recovery, matrix effect, and process efficiency were derived from the work of Matuszewski and coworkers (12). The recovery was calculated by dividing the peak area value of the extract of the spiked matrix by the peak area value of the spiked extract of a blank matrix. The matrix effect was tested by dividing the peak area value of the spiked extract of the blank matrix by the peak area value of the spiked extraction solution. The process efficiency was determined by dividing the peak area value of the extract of the spiked matrix by the peak area value of spiked extraction solution. Recovery, matrix effect, and process efficiency were all determined at three concentrations (low, medium, and high) in quintuplicate in a single run. To determine the dilution integrity, on three consecutive days, a QC sample with bedaquiline at a concentration of 12 mg/liter plasma was diluted 10 times and then analyzed in quintuplicate.

The stability of bedaquiline and M2 was tested under different test conditions, including storage stability and freeze-thaw stability at low- and high-level QC. The storage stability of bedaquiline and M2 was examined by storing QC samples at room temperature (20°C −25°C), in a refrigerator at 4°C, and after sample preparation in the autosampler at 10°C. Stability was also tested using five freeze-thaw cycles at −20°C. All stability tests were done using two different QC levels (low and high) in five determinations per concentration. Stability values were defined in terms of changes in concentration and should be ≤15%. Long-term sample stability was not tested, as data were provided by Janssen Pharmaceuticals.

Clinical application and compassionate-use program. Bedaquiline was made available in a compassionate-use program in The Netherlands before it became commercially available. Data were collected through review of the medical records of the patients who received bedaquiline within the compassionate-use program. Informed consent was obtained from the participants. The study was evaluated by the local ethics committee and was found to be in accordance with Dutch legislation due to its retrospective nature (METc 2013-491).

RESULTS

The mean retention time of bedaquiline was 1.9 min, the mean retention time of bedaquiline-D6 was also 1.9 min, and the mean retention time of M2 was 1.8 min (Fig. 2). Examining the selectivity of this analytical method, no interfering peaks from endogenous substances were observed at the retention time of bedaquiline or M2 in any of the six different lots of human serum (Fig. 2).

The calibration curves were linear over a range of 0.05 (LLOQ) to 6.00 mg/liter, and the correlation coefficient (R^2) values were 0.997 for bedaquiline and 0.999 for M2. The calibration curve parameters are presented in Table 3. The calculated accuracy ranged from 1.9% to 13.6% for bedaquiline and 2.9% to 8.5% for M2. Within-run precision ranged from 3.0% to 7.2% for bedaquiline and 3.1% to 5.2% for M2, and between-run precision ranged from 0.0% to 4.3% for bedaquiline and 0.0% to 4.6% for M2. The results of the accuracy and precision determinations for all QC levels for bedaquiline and M2 are shown in Table 3.

Recovery, matrix effect, and process efficiency data for bedaquiline and M2 are displayed in Table 1.

Results of determinations of the stability of bedaquiline and M2 under different test conditions are shown in Table 4. Measured (low and high) concentrations of QC samples for the freeze-thaw stability biased between 7.2% and 9.0% for bedaquiline and between 7.7% and 8.0% for M2, compared to the initial concentrations. Stability was determined by measuring QC samples stored at 4°C, and data differed by 1.1% to 4.4% from the nominal concentrations. After storage at room temperature for 72 h, the concentrations biased from −11.6% to −6.6% for bedaquiline and −7.8% to 0.5% for M2, compared to the initial concentrations. After sample preparation, the concentrations stored in the autosampler for 240 h biased between 3.5% and 6.7% for bedaquiline and between 2.0 and 2.6% for M2 from the nominal concentrations. Samples were stable for >1 year at −20°C for bedaquiline and M2 (data on file).

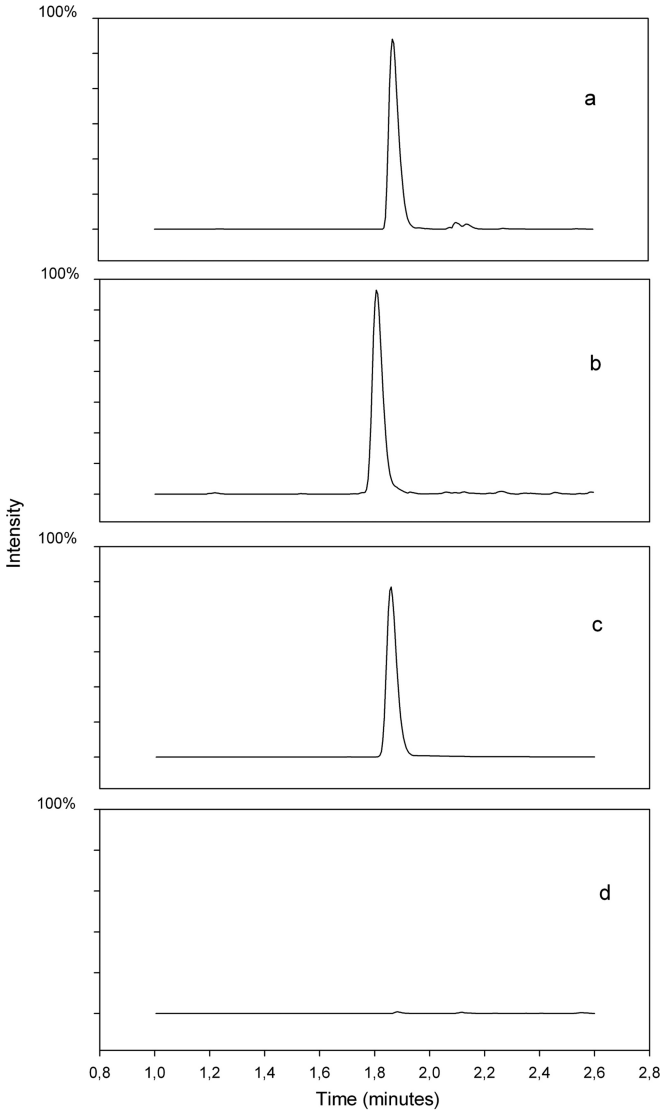

FIG 2 Chromatogram of bedaquiline, M2, and D6-bedaquiline. Chromatogram at lowest limit of quantitation: a, bedaquiline; b, M2; c, D6-bedaquiline, d, blank serum.

Clinical application; compassionate-use program. A 31-year-old female who resided in a highly TB-burdened country presented with fever despite having received two short courses of antimicrobial drugs. Her chest X-ray revealed characteristic signs of TB. *M. tuberculosis* was isolated from the sputum, revealing resistance to isoniazide, rifampin, ethambutol, and moxifloxacin.

TABLE 3 Calibration lines

Compound	*y* intercept (± SD)	Slope (± SD)	Correlation coefficient	Regression coefficient
Bedaquiline	−0.000170 ± 0.000166	0.0699 ± 0.00120	0.99676	0.99352
M2	0.00132 ± 0.000634	0.487 ± 0.00460	0.99902	0.99804

She was started on a regimen containing pyrazinamide, kanamycin, linezolid, and prothionamide. Nausea and vomiting necessitated replacement of prothionamide by cycloserine. After 4 months of treatment, bedaquiline, provided in a compassionate-use program, was added to optimize the treatment regimen. As part of routine supportive care, the MDR-TB treatment regimen was further optimized based on MIC testing and measurements of drug serum concentrations. The concentration-time profiles of bedaquiline and M2 can be observed in Fig. 3. Bedaquiline drug concentrations at the end of treatment were higher than the values previously reported at week 8 (13). M2 values were low, as expected (3). No drug-drug interactions were expected as no CYP3A4 inhibitors were coadministered. As the treatment was nearly completed and, more importantly, the patient tolerated the drug very well, no dose adjustments were made.

DISCUSSION

This report describes the design and validation of a simple and rapid analysis method using a triple-quadrupole LC-MS/MS system for the quantification of bedaquiline and its metabolite M2 in human plasma and deuterated bedaquiline as the internal standard. This LC-MS/MS method showed good accuracy and precision. The calibration curve was linear within a range of 0.050 (LLOQ) to 6.00 mg/liter and was found to be in accordance with the expected bedaquiline and M2 concentrations in serum (11, 13).

Compared to the analytical range of the procedure described by Rustomjee and coworkers (11), the analytical range of our procedure had an elevated higher limit of quantitation (HLQ) (2.0 versus 6.0 mg/liter). This may be advantageous, as dilution of the sample and subsequent rerun of a diluted sample become superfluous. However, our LLOQ was a little higher. This was of no clinical relevance, as the LLOQ of our procedure was designed for patients on bedaquiline treatment.

As already mentioned, a major advantage of this LC-MS/MS method is that it uses a simple protein precipitation which makes it suitable for PK monitoring. The two ICP-MS methods described earlier used liquid-liquid extraction and solid-phase extraction and were specifically designed for metabolite profiling and not for PK monitoring (9, 10). The run time is very short since the retention time of bedaquiline is 1.9 min. This facilitates high sample throughput. This is in line with the strategy of the World Health Organization designed to increase the capacity of high-quality laboratory systems using modern diagnostics for early, rapid, and accurate detection of TB, as it demands only one LC-MS/MS system to support a clinical therapeutic drug monitoring (TDM) service (14). In addition, bedaquiline sample stability is sufficient to enable transportation to a centralized laboratory. Recently, the first results of an international proficiency testing program for measurement of tuberculosis drugs were presented (15). Programs for drug susceptibility testing have been around for many years (16), but programs for measuring tuberculosis drug plasma concentrations have not. Participation in such an interlaboratory program provides additional quality assurance on top of intralaboratory QC monitoring, ensuring that plasma drug concentrations are measured accurately. In our opinion, bedaquiline should be used in such a program when plasma levels are measured frequently.

Since the antimicrobial action of bedaquiline is exposure dependent (2, 3), it is necessary, as with any drug with exposure-dependent killing characteristics, that target exposures are reached. Current data from animal studies on optimal drug exposure have not yet been translated to and validated in human studies. Due to the lack of clinical phase III data, including intensive

TABLE 4 Stability analysis results

Criterion (time of determination)	% bias at indicated QC concn level	
	Low	High
Autosampler stability (240 h)		
Bedaquiline	6.7	3.5
M2	2.6	2.0
Benchtop stability (72 h)		
Bedaquiline	−11.6	−6.6
M2	−7.8	0.5
Freeze-thaw stability (after 5 freeze-thaw cycles)		
Bedaquiline	7.2	9.0
M2	7.7	8.0
Stability at 4°C (240 h)		
Bedaquiline	1.4	1.2
M2	2.7	4.4

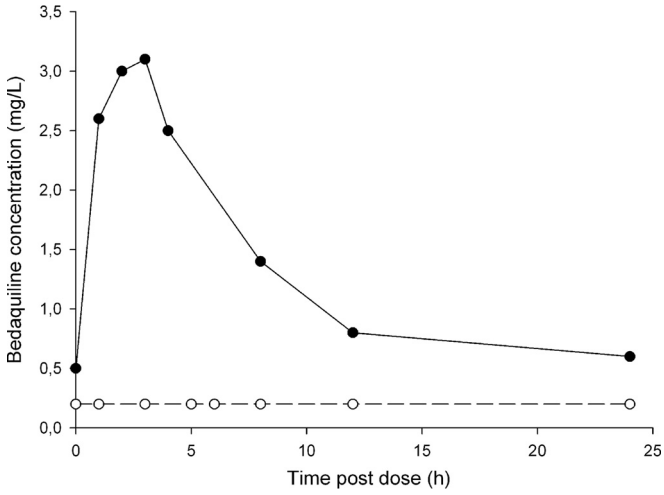

FIG 3 Bedaquiline concentration time curve. Solid circles represent bedaquiline; open circles represent M2.

PK sampling, MIC evaluation, and clinical outcome data, no clear target can be given. Hopefully, the data from the STREAM trial (NCT02409290) will provide relevant data. An average steady-state plasma concentration of 0.6 mg/liter was observed in patients who received bedaquiline in addition to a background regimen, showing a culture conversion rate higher than that seen with patients who received a placebo (13). To attain this target in patients suspected to have altered pharmacokinetics due to reduced food intake, drug-drug interactions, or altered disease state, bedaquiline serum concentration measuring may be helpful. We should try to optimize treatment in order to prevent treatment failure, especially in patients infected with extensively resistant *M. tuberculosis* strains with few treatment options left (17).

For first-line treatment, it has already been shown that drug exposure predicts outcomes (6). In cases of treatment of MDR-TB, that conclusion is less clear, predominantly due to a lack of data. A recent study showed that fluoroquinolone resistance developed in over 10% of the patients studied (18), likely because the pharmacokinetic-pharmacodynamic targets were not reached in a substantial proportion of the patients (19). Recent phase III studies evaluating new strategies for treatment of TB did not include measurements of drug concentrations as a covariate in the analysis of the primary outcome of the study (20–22). As evaluation of drug concentrations in relation to the susceptibility of the pathogen may help to explain differences in treatment responses, it is highly desirable that pharmacokinetics should become part of future phase III studies (23). Therefore, plasma drug concentrations of new drugs such as bedaquiline and delamanid will receive more attention in future studies as well as in daily practice. Also, in cases of off-label use or in studies evaluating the bactericidal activity of drug combinations, information on drug exposure is helpful to explain treatment outcome (24–26).

The described method was developed for monitoring bedaquiline serum concentrations in daily practice because it meets the criteria for TDM but is also suitable for clinical pharmacokinetic studies or clinical trials to further investigate the use of bedaquiline in specific TB patient groups suffering from comorbidities or drug-drug interactions.

Conclusion. A simple LC-MS/MS method to quantify bedaquiline and M2 in human serum using deuterated bedaquiline as the internal standard has been developed and validated. This method can be used in daily practice and in clinical studies evaluating drug exposure in relation to clinical outcome.

ACKNOWLEDGMENTS

We thank Janssen Pharmaceuticals for providing the bedaquiline, M2, and deuterated internal standard.

J.-W.C.A., O.A., W.D.L., and T.V.D.W. participated in a Janssen Pharmaceuticals advisory board meeting.

REFERENCES

1. **Cox E, Laessig K.** 2014. FDA approval of bedaquiline—the benefit-risk balance for drug-resistant tuberculosis. N Engl J Med **371:**689–691. http://dx.doi.org/10.1056/NEJMp1314385.
2. **Andries K, Verhasselt P, Guillemont J, Göhlmann HWH, Neefs J-M, Winkler H, Van Gestel J, Timmerman P, Zhu M, Lee E, Williams P, de Chaffoy D, Huitric E, Hoffner S, Cambau E, Truffot-Pernot C, Lounis N, Jarlier V.** 2005. A diarylquinoline drug active on the ATP synthase of Mycobacterium tuberculosis. Science **307:**223–227. http://dx.doi.org/10.1126/science.1106753.
3. **Rouan MC, Lounis N, Gevers T, Dillen L, Gilissen R, Raoof A, Andries K.** 2012. Pharmacokinetics and pharmacodynamics of TMC207 and its N-desmethyl metabolite in a murine model of tuberculosis. Antimicrob Agents Chemother **56:**1444–1451. http://dx.doi.org/10.1128/AAC.00720-11.
4. **van Heeswijk RPG, Dannemann B, Hoetelmans RMW.** 2014. Bedaquiline: a review of human pharmacokinetics and drug-drug interactions. J Antimicrob Chemother **69:**2310–2318. http://dx.doi.org/10.1093/jac/dku171.
5. **Pasipanodya JG, Srivastava S, Gumbo T.** 2012. Meta-analysis of clinical studies supports the pharmacokinetic variability hypothesis for acquired drug resistance and failure of antituberculosis therapy. Clin Infect Dis **55:**169–177. http://dx.doi.org/10.1093/cid/cis353.
6. **Pasipanodya JG, McIlleron H, Burger A, Wash PA, Smith P, Gumbo T.** 2013. Serum drug concentrations predictive of pulmonary tuberculosis outcomes. J Infect Dis **208:**1464–1473. http://dx.doi.org/10.1093/infdis/jit352.
7. **Svensson E, Murray S, Karlsson M, Dooley K.** 2015. Rifampicin and rifapentine significantly reduce concentrations of bedaquiline, a new anti-TB drug. J Antimicrob Chemother **70:**1106–1114.
8. **World Health Organization.** 2013. The use of bedaquiline in the treatment of multidrug-resistant tuberculosis Interim policy guidance, p 1–57. World Health Organization, Geneva, Switzerland.
9. **Cuyckens F, Balcaen LIL, De Wolf K, De Samber B, Van Looveren C, Hurkmans R, Vanhaecke F.** 2008. Use of the bromine isotope ratio in HPLC-ICP-MS and HPLC-ESI-MS analysis of a new drug in development. Anal Bioanal Chem **390:**1717–1729. http://dx.doi.org/10.1007/s00216-007-1761-6.
10. **Balcaen LIL, De Samber B, De Wolf K, Cuyckens F, Vanhaecke F.** 2007. Hyphenation of reverse-phase HPLC and ICP-MS for metabolite profiling—application to a novel antituberculosis compound as a case study. Anal Bioanal Chem **389:**777–786. http://dx.doi.org/10.1007/s00216-007-1303-2.
11. **Rustomjee R, Diacon AH, Allen J, Venter A, Reddy C, Patientia RF, Mthiyane TCP, De Marez T, Van Heeswijk R, Kerstens R, Koul A, De Beule K, Donald PR, McNeeley DF.** 2008. Early bactericidal activity and pharmacokinetics of the diarylquinoline TMC207 in treatment of pulmonary tuberculosis. Antimicrob Agents Chemother **52:**2831–2835. http://dx.doi.org/10.1128/AAC.01204-07.
12. **Matuszewski BK, Constanzer ML, Chavez-Eng CM.** 2003. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal Chem **75:**3019–3030. http://dx.doi.org/10.1021/ac020361s.
13. **Diacon AH, Pym A, Grobusch M, Patientia R, Rustomjee R, Page-Shipp L, Pistorius C, Krause R, Bogoshi M, Churchyard G, Venter A, Allen J, Palomino JC, De Marez T, van Heeswijk RPG, Lounis N, Meyvisch P, Verbeeck J, Parys W, de Beule K, Andries K, Mc Neeley DF.** 2009. The diarylquinoline TMC207 for multidrug-resistant tuberculosis. N Engl J Med **360:**2397–2405. http://dx.doi.org/10.1056/NEJMoa0808427.
14. **Srivastava S, Peloquin CA, Sotgiu G, Migliori GB.** 2013. Therapeutic drug management: is it the future of multidrug-resistant tuberculosis treatment? Eur Respir J **42:**1449–1453. http://dx.doi.org/10.1183/09031936.00073213.
15. **Aarnoutse RRE, Sturkenboom MG, Robijns K, Harteveld AR, Greijdanus B, Uges DR, Touw DJ, Alffenaar JW.** 16 April 2015. An interlaboratory quality control programme for the measurement of tuberculosis drugs. Eur Respir J http://dx.doi.org/10.1183/09031936.00177014.
16. **Angra PK, Taylor TH, Iademarco MF, Metchock B, Astles JR, Ridderhof JC.** 2012. Performance of tuberculosis drug susceptibility testing in U.S. laboratories from 1994 to 2008. J Clin Microbiol **50:**1233–1239. http://dx.doi.org/10.1128/JCM.06479-11.
17. **Lange C, Abubakar I, Alffenaar J-WC, Bothamley G, Caminero JA, Carvalho ACC, Chang K-C, Codecasa L, Correia A, Crudu V, Davies P, Dedicoat M, Drobniewski F, Duarte R, Ehlers C, Erkens C, Goletti D, Günther G, Ibraim E, Kampmann B, Kuksa L, de Lange W, van Leth F, van Lunzen J, Matteelli A, Menzies D, Monedero I, Richter E, Rüsch-Gerdes S, Sandgren A, Scardigli A, Skrahina A, Tortoli E, Volchenkov G, Wagner D, van der Werf MJ, Williams B, Yew W-W, Zellweger J-P, Cirillo DM.** 2014. Management of patients with multidrug-resistant/extensively drug-resistant tuberculosis in Europe: a TBNET consensus statement. Eur Respir J **44:**23–63. http://dx.doi.org/10.1183/09031936.00188313.
18. **Cegielski J, Dalton T, Yagui M, Wattanaamornkiet W, Volchenkov G, van der Walt M, Tupasi T, Kcasnovsky C, Kuznetsiova T, Kurbutova E,**

Kummik T, Kuksa L, Kliiman K, Kiryanova E, Kim H, Kim C, Kazennyy B, Jou R, Huang W, Ershova J, Erokin V, Diem L, Contreras C, Chio S, Chernousova L, Chen M, Caoili J, Bayona J, Akksilp S, Global Preserving Effective TB Treatment Study (PETTS) Investigators. 2014. Extensive drug resistance acquired during treatment of multidrug-resistant tuberculosis. Clin Infect Dis **59:**1049–1063. http://dx.doi.org/10.1093/cid/ciu572.

19. **Alffenaar J-WC, Gumbo T, Aarnoutse RE.** 2015. Acquired drug resistance: we can do more than we think! Clin Infect Dis **60:**969–970.
20. **Merle CS, Fielding K, Sow OB, Gninafon M, Lo MB, Mthiyane T, Odhiambo J, Amukoye E, Bah B, Kassa F, N'Diaye A, Rustomjee R, de Jong BC, Horton J, Perronne C, Sismanidis C, Lapujade O, Olliaro PL, Lienhardt C.** 2014. A four-month gatifloxacin-containing regimen for treating tuberculosis. N Engl J Med **371:**1588–1598. http://dx.doi.org/10.1056/NEJMoa1315817.
21. **Jindani A, Harrison TS, Nunn AJ, Phillips PPJ, Churchyard GJ, Charalambous S, Hatherill M, Geldenhuys H, McIlleron HM, Zvada SP, Mungofa S, Shah NA, Zizhou S, Magweta L, Shepherd J, Nyirenda S, van Dijk JH, Clouting HE, Coleman D, Bateson ALE, McHugh TD, Butcher PD, Mitchison DA.** 2014. High-dose rifapentine with moxifloxacin for pulmonary tuberculosis. N Engl J Med **371:**1599–1608. http://dx.doi.org/10.1056/NEJMoa1314210.
22. **Gillespie SH, Crook AM, McHugh TD, Mendel CM, Meredith SK, Murray SR, Pappas F, Phillips PPJ, Nunn AJ.** 2014. Four-month moxifloxacin-based regimens for drug-sensitive tuberculosis. N Engl J Med **371:**1577–1587. http://dx.doi.org/10.1056/NEJMoa1407426.
23. **Warner DF, Mizrahi V.** 2014. Shortening treatment for tuberculosis—back to basics. N Engl J Med **371:**1642–1643. http://dx.doi.org/10.1056/NEJMe1410977.
24. **Diacon AH, Dawson R, von Groote-Bidlingmaier F, Symons G, Venter A, Donald PR, van Niekerk C, Everitt D, Hutchings J, Burger DA, Schall R, Mendel CM.** 2015. Bactericidal activity of pyrazinamide and clofazimine alone and in combinations with pretomanid and bedaquiline. Am J Respir Crit Care Med **191:**943–953. http://dx.doi.org/10.1164/rccm.201410-1801OC.
25. **Grossman T, Shoen C, Jones S, Jones P, Cynamon M, Locher C.** 2015. The efflux pump inhibitor timcodar improves the potency of antimycobacterial agents. Antimicrob Agents Chemother **59:**1534–1541. http://dx.doi.org/10.1128/AAC.04271-14.
26. **Philley J, Wallace RJ, Jr, Benwill JL, Taskar V, Brown-Elliott BA, Thakkar F, Aksamit TR, Griffith DE.** 12 February 2015, posting date. Preliminary results of bedaquiline as salvage therapy for patients with nontuberculous mycobacterial lung disease. Chest http://dx.doi.org/10.1378/chest.14-2764.